



Genetic and morphological heterogeneity within *Eucyclops serrulatus* (Fischer, 1851) (Crustacea: Copepoda: Cyclopidae)

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Numerous studies have revealed 17 species and subspecies in the species complex *E. serrulatus* (Fischer, 1851). As a result, it is clear now that the former cosmopolitan species in fact represent a group of closely related species. Some of them are possibly cryptic taxa and need to be redescribed. The objective of this study was to analyse the broadly distributed *E. serrulatus* populations in Europe and Asia: in Saint Petersburg (the type locality, Russia), Central Russia, Odessa region and Zakarpattia region – Dniester and Danube river drainage basins accordingly (Ukraine), Valencia (Spain), Oslo (Norway), Paris (France) and in Taiwan. Mitochondrial gene (CO1) and morphological analyses revealed a significant heterogeneity between and within the above-mentioned populations. Populations from Ukraine displayed 27.1% genetic differences, supported by qualitative and quantitative morphological distinctions. The heterogeneity in the CO1 gene was shown even in *E. serrulatus* from the Orlov Pond (Russia) – *terra typica* for this species. Morphological analysis did not confirm this heterogeneity. Valencian (Spain) and Taiwanese *E. cf. serrulatus* separate out from other populations. As a result, a new species *Eucyclops taiwanensis* sp. n. from Taiwan is described.

<http://www.zoobank.org/urn:lsid:zoobank.org:pub:237198AE-4549-4E55-A836-AAE8A039CB51>

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Introduction

The *serrulatus* group of the cyclopoid freshwater genus *Eucyclops* (Copepoda: Cyclopoida) comprises species having 12-segmented antennules with the three most distal segments possessing a smooth hyaline membrane, caudal rami with average length (l/w ratio 3.5–4), a strong inner spine on P5 and a number of microcharacters presented on the antennary basipodite and the caudal surface of P4 coxopodite, as defined by Alekseev et al. (2006).

The type species of this genus is *Eucyclops serrulatus* (Fischer). This taxon was discovered in the mid-nineteenth century in a pond at Peterhof close to Saint Petersburg, Russia (Fischer 1851). Over the past century the species has been reported from water bodies around the world (Dussart and Defaye 1985, 2006) and in consequence *E. serrulatus* has long been considered a cosmopolitan species.

In recent years the distributional area of the species was revised and restricted to a supposed Palearctic distribution. All records of the species in Australia, North

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America and other zoogeographical regions are, possibly, the result of recent invasions (for instance with ballast water of ships) (Alekseev and Defaye 2011).

The name ‘*serrulatus* group’ is used for the *E. serrulatus* species complex and it was established by Kiefer in 1928 for a group of tropical species closely related to *E. serrulatus* (Alekseev and Defaye 2011). The *serrulatus* group now includes 17 species and subspecies, which differ from each other in the presence of microcharacters of the antennary basipodite and the P4 coxopodite (Dussart 1984; Alekseev 1990, 2000, 2008, 2010; Reid 1992; Ishida 1997, 1998, 2000, 2001, 2003).

Despite all efforts to establish the taxonomic composition of the *serrulatus* group, and the recent detailed redescription of *E. serrulatus* (Alekseev et al. 2006), there are still many questions to solve: the group is truly a group of cryptic species that differ only by microcharacters and often by DNA sequences. As a result, incorrect species identification often occurs: new species descriptions have been published, but their taxonomical status has subsequently been revised, or still await revision, e.g. *E. roseus* described by Ishida (1997) in Japan is in fact a subspecies of *E. agiloides* according to Alekseev and Defaye (2011); the status of *E. miracleae* should be revised because its morphological traits correspond to *E. agiloides* (Alekseev and Defaye 2011).

Surveys of new localities reveal new species, subspecies and forms of *E. serrulatus*. A study of mountain populations in Eastern Europe (Hamrova et al. 2012) has revealed a large genetic heterogeneity in mitochondrial genes 12SrRNA and Cytb. Nucleotide differences between some clades reached 50% (Hamrova et al. 2012).

Moreover, the species *E. serrulatus* from the type locality is represented by three morphological forms (types): A, B and C. These forms have morphological differences in the caudal setae, swimming legs and pore signature, but genetic analysis of the 18S rRNA gene does not reveal significant differences. Moreover, no reproductive isolation was found among these types. The same population structure of *E. serrulatus* has been shown in Siberia (Tumen) and Western Europe (Ghent) (Alekseev et al. 2006).

The objective of this study was to analyse the broadly distributed *E. serrulatus* populations in Europe and Asia. The origin of this work is largely based on hybridization experiments of *E. serrulatus* populations from the multiple localities of Ukraine (the Danube, Dnieper and Dniester river drainage basins) and Russia (Saint Petersburg and Leningrad region) (Monchenko et al. 2011). It was shown there is reproductive isolation between the populations from the river Danube, Dnieper and Dniester drainage basins whereas until recently it was thought that this territory was inhabited by a single species, *E. serrulatus*. *Eucyclops cf. serrulatus* from Taiwan was also previously observed and preliminary morphological searches showed new species presence (Alekseev and Defaye 2011).

Material and methods

Population sampling

Samples of *Eucyclops* for molecular-genetic and morphological analyses were collected in Orlov Pond (*terra typica* for *Eucyclops serrulatus*) and in the Ut' River by N. Sukhikh; the material from the pond at Golubinoe village from the Zakarpattia region and from the Dniester Liman was sampled by Larisa V. Samchishina; and specimens from the Tavricheskii Pond, the pond in Oslo, Creteil Lake, the Xucar River, Abvill Pond and Lu-Shad Reservoir were collected by V. Alekseev (Table 1, Figure 1).

Table 1. *Eucyclops* sampling sites used for molecular-genetic* and morphological† studies.

No	Country, area	Locality	Coordinates	Date
1	Russia, Saint Petersburg	Tavricheskii Pond*†	59.9461°N, 30.3733°W	May 2010
1	Russia, Saint Petersburg	Orlov Pond*†	59.8536°N, 30.0597°W	November 2010
2	Russia, Udmurtia	Ut' River*	57.7069°N, 52.4969°W	July 2010
3	Ukraine, Odesskii region	Dniester Liman*†	46.2519°N, 30.355°W	August 2010
4	Ukraine, Zakarpattia region	Pond of Golubinoe village*†	48.5714°N, 22.9697°W	August 2010
5	Norway, Oslo	Pond*	59.9111°N, 10.7528°W	June 2009
6	France, Paris	Creteil Lake*	48.8333°N, 2.3333°W	April 2010
7	Spain, Valencia	Xucar River*	39.4667°N, 0.375°W	July 2009
8	Taiwan	Lu-Shad Reservoir*	25.0333°N, 121.6333°W	October 2009
8	Taiwan	Abvill Pond*	25.0333°N, 121.6333°W	October 2009

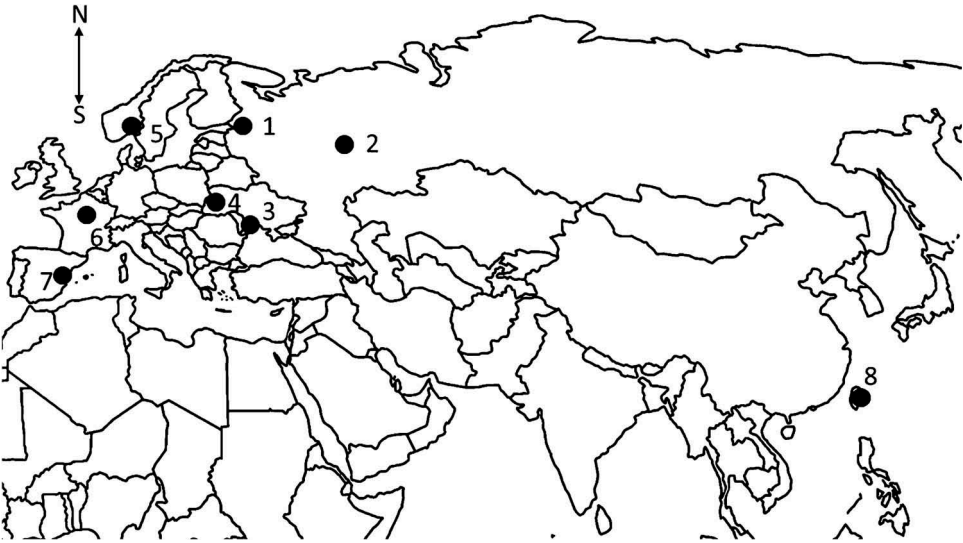


Figure 1. The localities of the *Eucyclops serrulatus* (Fischer) and *Eucyclops taiwanensis* sp. n., populations studied. Locality names are listed in Table 1.

All samples were collected by horizontal trawl at 1 m depth in near-shore areas using a standard plankton net with 100- μ m mesh size. Organisms in these samples were conserved in 96% alcohol. For the morphological survey only, we used an extensive collection of *Eucyclops serrulatus* (V. Alekseev responsible) from the Urals, Siberia (Russia), Slovenia, and Belgium. These local populations were represented by 1–5 individuals.

Molecular-genetic analysis

Mitochondrial cytochrome C oxidase I (COI) fragment was used in our analysis. For each individual, a 651 bp fragment of the mtCOI gene was sequenced, but for individuals from France and Spain 359 bp sequences were obtained. A total of 37 adult specimens were studied with this fragment of the mtCOI gene: 10 specimens from Orlov Pond, one from the Ut' River, five from the pond in Golubinoe village from the Zakarpattia region, five from the Dniester Liman, two from Tavricheskii Pond, three from the pond in Oslo, two from the Creteil Lake, three from the Xucar River and five from Taiwan.

Genomic DNA was extracted from copepods preserved in 96% ethanol using the standard methods described by Aljanabi and Martinez (1997) or with Diatom™ DNA Prep 200 Kit (Laboratory Izogen, Moscow, Russia).

Universal primers COIH 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and COIL 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'; Folmer et al. 1994) were used for polymerase chain reaction (PCR) of COI amplification. PCR conditions started with DNA denaturing at 95°C (60 s), followed by 35 cycles of 30 s denaturing at 95°C, 30 s annealing at 50°C, and 50 s extension at 72°C, and then a final extension at 72°C for 7 min. The product was purified with a Qiaquick PCR purification kit (Qiagen,

Table 2. The CO1 sequences deposited in GenBank.

Species	GenBank accession numbers
<i>Eucyclops serrulatus</i> (Fischer)	KC627297-323, JF520831
<i>Eucyclops taiwanensis</i> sp. n.	KC627324-330
<i>Eucyclops macruroides</i> (Lilljeborg)	KC627333
<i>Eucyclops macrurus</i> (Sars G.O.)	KC627334-337
<i>Eucyclops speratus</i> (Lilljeborg)	KC627338
<i>Macrocyclops albidus</i> (Jurine)	KC627343
<i>Megacyclops viridis</i> (Jurine)	KC627344-45
<i>Cyclops abyssorum</i> G. O. Sars	KC627287-90
<i>Cyclops insignis</i> Claus	KC627291-95
<i>Cyclops strenuus</i> Fischer	KC627296

Valencia, CA, USA) then sequenced using an Applied Biosystems Inc. 3130 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA).

Both chains were sequenced to confirm the accuracy of each haplotype sequence. Sequences were aligned using the algorithm CLUSTAL W (Thompson et al. 1994) implemented in BIOEDIT v.7.0.3 (Hall 1999) with manual editing of ambiguous sites. The obtained sequences were deposited in GenBank (Table 2).

Phylogenetic reconstructions were performed by maximum likelihood (ML) and by Bayesian analyses with MrBayes 3.1 (Ronquist and Huelsenbeck 2003). The best-fitting model under the ML criterion was selected using the Bayesian information criterion (BIC) and Akaike information criterion (AIC) output of jMODELTEST v0.1.1 (Posada 2008). The ML analyses were conducted using a TPM1uf model with gamma rate distribution (G) and without invariable sites (I), the best tree topology was searched using NNI and SPR methods and node stability was estimated by 100 nonparametric bootstrap replicates.

Two independent runs of 10 million generations each were performed for the Bayesian analyses. Analyses were undertaken by sampling every 1000th generation. The analyses were conducted also using a TPM1uf model with gamma rate distribution (G).

For the outgroup in tree construction, CO1 sequences were obtained for the following species: *Eucyclops macrurus* (Sars G.O.), *Eucyclops macruroides* (Lilljeborg), *Eucyclops speratus* (Lilljeborg), *Cyclops strenuus* Fischer, *Cyclops insignis* Fischer, *Cyclops abyssorum* (Sars G.O.), *Megacyclops viridis* (Jurine) and *Macrocyclops albidus* (Jurine) (Table 2). Two sequences of *E. serrulatus* from Italy KC986942.1 and KC986941.1 were retrieved from GenBank.

Morphological analysis

A total of 124 adult specimens were used for the investigations: 49 females and 12 males from Orlov Pond; 15 females and 15 males from Tavricheskii Pond in Saint Petersburg; nine females and six males from the Dniester Liman in the Odessa region;

five females from the Zakarpattia region in Ukraine; and 10 females and three males from Taiwan.

The samples were sorted under a dissection microscope (Olympus, SZX2, Tokyo, Japan). Before dissection, copepod adults were measured with an ocular micrometer (5 μm resolution). The dissection was processed in glycerol. After dissection, the specimens were placed on slides in pure glycerol, covered with a cover slip and ringed with Canadian balsam. The slides were then examined at maximum resolution up to 1000 \times (Plan objective 100 \times , oil immersion) under a compound microscope (Zeiss IMAGER, Oberkochen, Germany) equipped with Nomarski system for differential interference contrast microscopy and a drawing tube.

To study the populations both sexes were analysed. More than 20 different character measurements were performed, including measurements of caudal ramus structures, exo- and endopodite P4 structures and also secondary sexual dimorphic characters typically used in copepod taxonomy, as well as characters of the fifth legs (P5), spinules and setules on the coxopodite and intercoxal sclerite of P4 caudal surface, and micropatterns on caudal surface of the antennal basipodite (according to the numerical scheme illustrated in Alekseev et al. 2006).

After making measurements, index calculations were performed and the most informative indexes were chosen. The most informative characters used for the index calculations are shown below. Statistical analysis was performed with nonparametric Kruskal–Wallis test using standard statistical software Statistica 7. The significance level was set as $p < 0.05$.

The type material of *Eucyclops taiwanensis* sp. n. was deposited in the type collection of the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (ZIN RAN) under reference numbers 55090–55091.

Copepod body and appendage terminology mainly followed Huys and Boxshall (1991) and Alekseev and Defaye (2011). Abbreviations used are: caudal L, length of caudal ramus; caudal W, width of caudal ramus; caudal IN, innermost (VI) caudal seta; OUTseta, outermost (III) caudal seta; DORseta, lateral (II) caudal seta; P1–P4, swimming legs 1–4; P5, leg 5; Exo, exopodite; endo, endopodite; Lseg, segment length; Sp, spine.

Results

Patterns of genetic heterogeneity within *E. serrulatus*

Phylogenetic analysis of mitochondrial CO1 gene revealed four clades with deep splits among them with maximum pairwise divergences of 26.6–29.9%. (Figure 2, Table 3). All clades presented strong support, 100% as Bayesian posterior probabilities as well bootstrap percentages (for ML).

The two first major clades (1 and 2) of *E. serrulatus* mainly corresponded to geographical locations, which were analysed. The specimens from the Zakarpattia region belonged to clade 1 and the specimens from the Dniester Liman belonged to clade 2. The sequences from the pond in Oslo, Creteil Lake, from Tavricheskii Pond in Saint Petersburg and two Italian sequences retrieved from Genbank also belonged to clade 1. The sequence from the Ut' River belonged to clade 2. Sequences from the type population from Orlov Pond in Saint Petersburg separated into clades 1 and 2, and genetic differences between them were 27.1% of nucleotide differences.

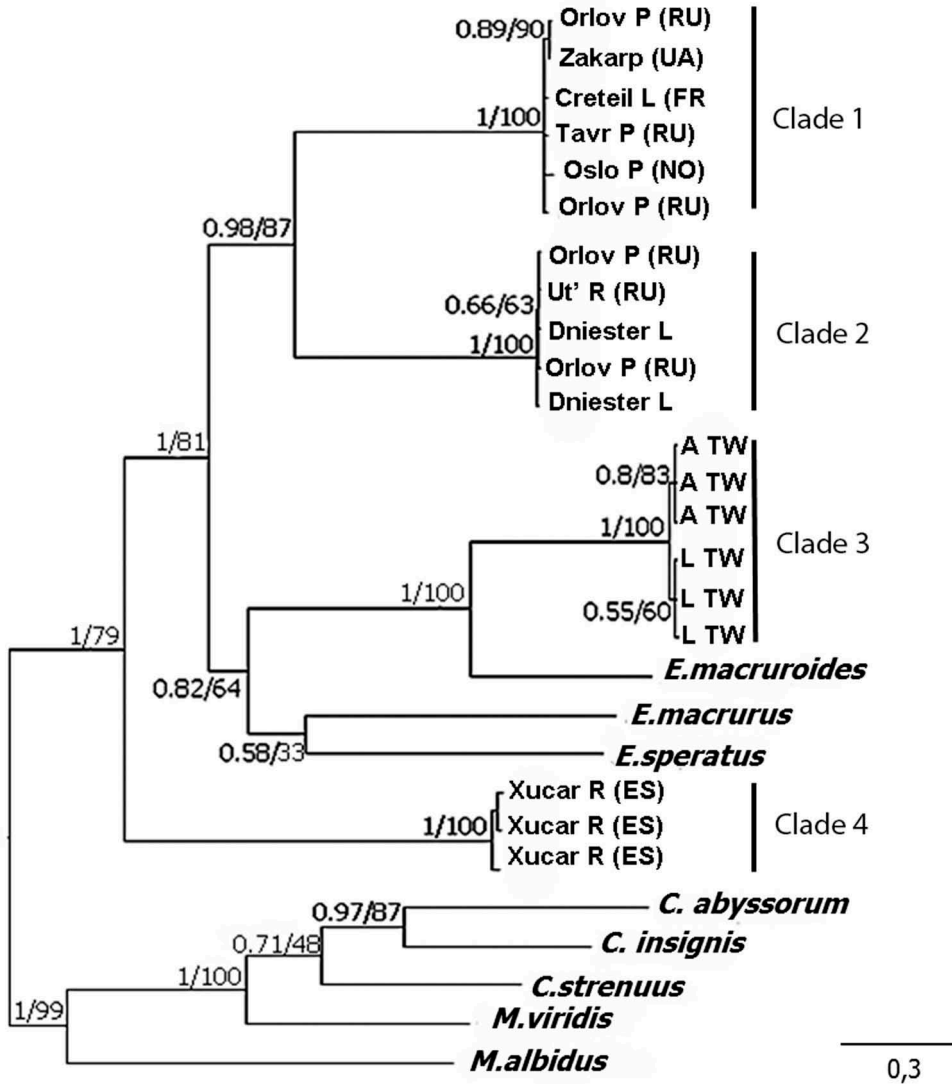


Figure 2. Phylogenetic tree constructed based on data from mitochondrial cytochrome c oxidase subunit I (COI) region (651 bp) by the Bayesian method using the TPM1uf +G model. Numbers beside nodes indicate Bayesian posterior probabilities (BPP) and bootstrap values. Clade 1 includes sequences of *Eucyclops serrulatus* (Fischer) from the Zakarpattia region of Ukraine, Orlov Pond and Tavrisheskii Pond in Saint Petersburg, Creteil Lake in Paris and the pond in Oslo; Clade 2: *Eucyclops serrulatus* sequences from the Dniester Liman in Ukraine, Orlov Pond in Saint Petersburg and Central Russia (Udmurtia). Clade 3: *Eucyclops taiwanensis* sp. n. sequences from ponds in Taiwan. Clade 4: *Eucyclops cf. serrulatus* sequences from the Xucar River in Spain.

Table 3. Mean between-group genetic divergences of the *Eucyclops cf. serrulatus* clades (according to Figure 2). The values are given for analysing fragments of the mitochondrial CO1 region. Divergences calculated with Tamura 3-parameter model.

	Clade 1	Clade 2	Clade 3
Clade 1			
Clade 2	27.1%		
Clade 3	28.5%	26.6%	
Clade 4	29.4%	29.9%	28.8%

Clades 3 and 4 corresponded to geographical locations. Clade 3 included specimens from the Taiwanese ponds and clustered with the sequence of *Eucyclops macruroides* (Lilljeborg, 1901). The specimens from the Xucar River formed clade 4 with maximal differences from other clades 28.8–29.9% (Table 4).

The mean sequence divergence within clades was not more than 0.8% and for sequences from Taiwan only this value was 1.2%. There was no overlap in haplotypes among the different studied regions, except at the type locality in Saint Petersburg.

Morphological analysis of the studied *Eucyclops* populations

Study of the Ukrainian populations

A total of more than 20 measurements of thoracic legs and caudal rami were carried out and 16 indexes were calculated; eight of them were statistically reliable ($p < 0.05$) for the studied populations.

First an analysis of the Zakarpattia and the Dniester Liman populations from Ukraine was done. The study of the population from Zakarpattia was carried out only in females, whereas both sexes were included in the Dniester population study.

One index was distinguished reliably between these populations: the ratio of the innermost caudal seta (VI)/outermost caudal seta (III) (IN/OUTseta): 1.5 ± 0.0 for Dniester *E. serrulatus* and 1.4 ± 0.0 for *E. serrulatus* from the Zakarpattia region (Table 4, Figure 3). However, considerable qualitative differences divided these populations: the length of the setules on the edge of the P4 intercoxal sclerite and the micropatterns presented on the caudal surface of the antennary basipodite (Figure 3A–D). The setules on the P4 intercoxal sclerite in Dniester individuals (both in males and females) are absent or short and arranged at an angle to the sclerite (Figure 3A) whereas the Zakarpattia population possesses thick hair-like setae with a length about 1/3 of the coxal spine (Figure 3B). The micropatterns on the antennal basipodite caudal (posterior) surface differ mainly in groups 1, 2, 5 and 17 as shown in Figure 3C, D. The groups of Dniester individuals have more setules and the setules are longer compared with the Zakarpattia population.

Study of the type population from Orlov Pond in Saint Petersburg (Russia)

The same characters were used to test the type populations from Orlov and Tavricheskii ponds; these populations did not differ morphologically. Both qualitative and quantitative characters used to separate the Ukrainian populations were also

Table 4. Morphometric indexes in females *Eucyclops serrulatus* (Fischer) and *Eucyclops taiwanensis* sp. n. Mean \pm error of the mean (min–max). Feature abbreviations shown in Figure 3.

	Cauda, L/W	Cauda, IN/OUTseta	Cauda, IN/DORseta	P4 exo Lseg/Sp1	P4 endo Sp1/Lseg	P4 endo Inseta/Lseg
Saint Petersburg	4.7 \pm 0.1 (3.3–6.5)	1.2 \pm 0.0 (0.8–1.6)	1.7 \pm 0.1 (1.4–2.6)	1.2 \pm 0.0 (0.7–1.7)	1.0 \pm 0.0 (0.9–1.1)	0.8 \pm 0.0 (0.7–1.3)
Dniester Liman	4.6 \pm 0.2 (4.5–5.6)	1.5 \pm 0.0 (1.4–1.7)	1.9 \pm 0.1 (1.5–2.4)	1.5 \pm 0.1 (1.2–2.1)	0.9 \pm 0.0 (0.8–1.1)	0.8 \pm 0.1 (0.6–0.9)
Zakarpattia region	5.1 \pm 0.2 (4.7–5.4)	1.4 \pm 0.0 (1.3–1.4)	1.8 \pm 0.0 (1.7–1.9)	1.4 \pm 0.0 (1.3–1.4)	1.0 \pm 0.0 (0.9–1.0)	0.9 \pm 0.0 (0.9–1)
Taiwan	4.2 \pm 0.0 (4.0–4.5)	1.3 \pm 0.0 (1.1–1.4)	1.3 \pm 0.0 (1.2–1.4)	1.2 \pm 0.0 (1.1–1.3)	1.1 \pm 0.0 (0.8–1.1)	1.1 \pm 0.0 (0.9–1.2)

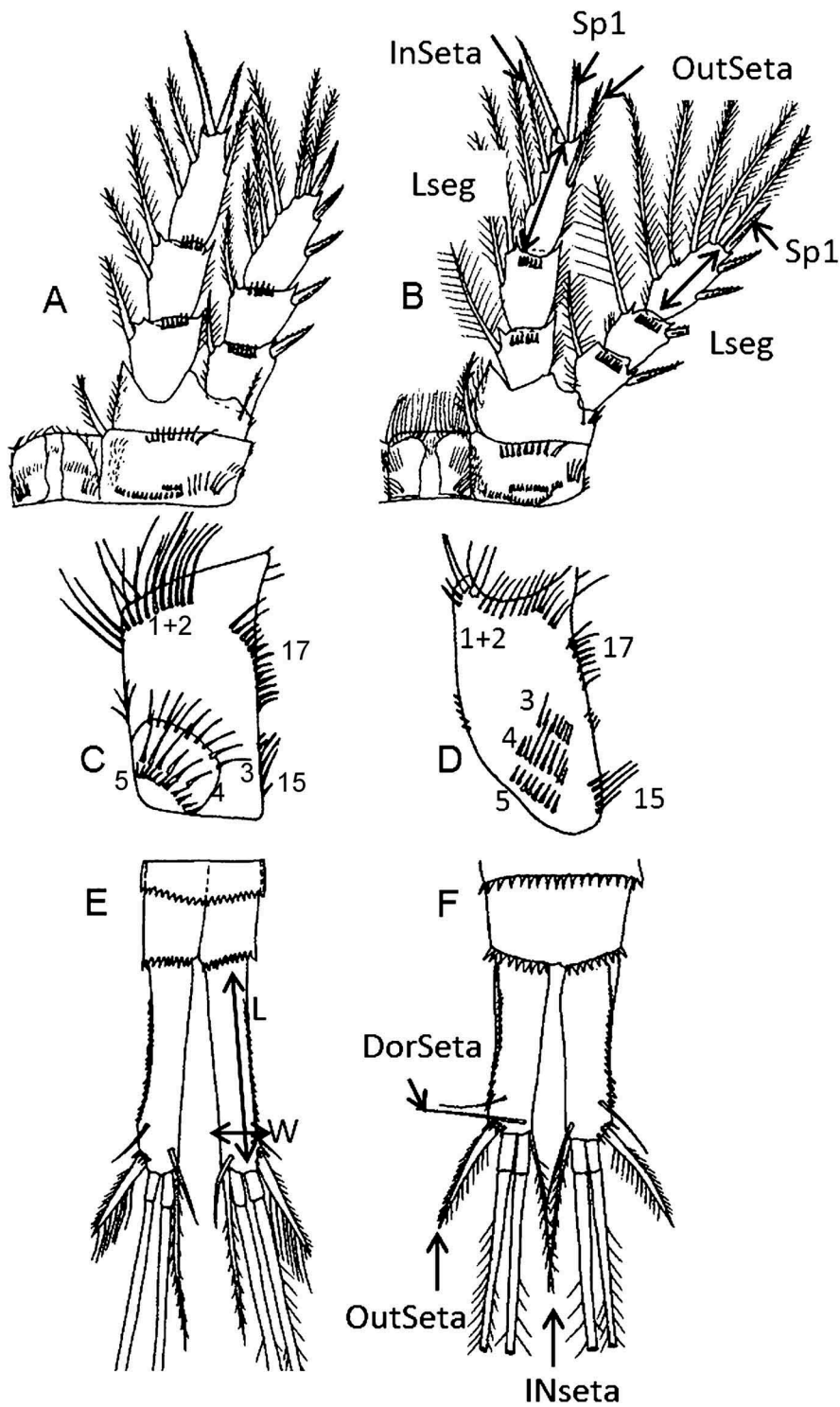


Figure 3. *Eucyclops serrulatus* (Fischer) from the Dniester Liman (A, C, E) and Zakarpattia regions (B, D, F) of Ukraine. (A, B) P4, caudal side with feature abbreviations used; (C, D) antenna, caudal side; (E, F) caudal ramus with abbreviations.

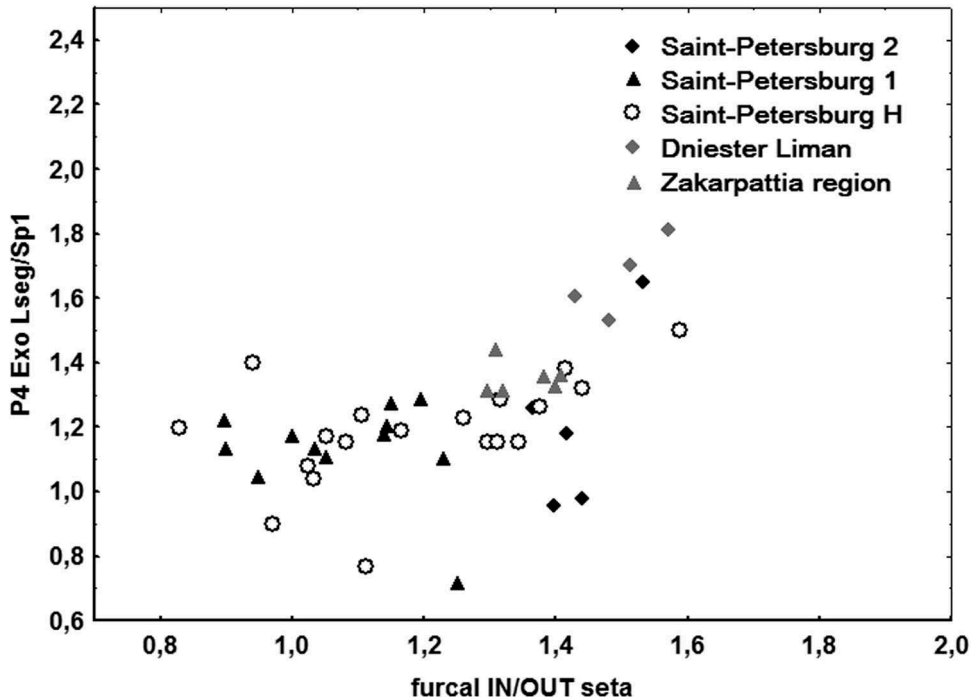


Figure 4. Distribution of *Eucyclops serrulatus* (Fischer) females from different localities on the base of caudal rami index INseta/OUTseta, and P4 exo Lseg/Sp1 (see Table 4 and text). Populations from Dniester Liman \blacklozenge ; Zakarpattia region \blacktriangle ; and from the type locality in Orlov Pond, Saint Petersburg: individuals from Orlov Pond resembling the Zakarpattia population \blacktriangle ; individuals from Orlov Pond resembling Dniester Liman \blacklozenge and individuals from Orlov Pond \circ looking like hybrids between the two Ukrainian populations.

analysed for the type population but this set of characters was mostly not useful for discrimination in the type population.

Most of the studied individuals (males and females) from Orlov Pond, Saint Petersburg possessed the qualitative characters of both Ukrainian populations. For instance, they had the short setules on the P4 intercoxal sclerite arranged at an angle to the sclerite like in Dniester individuals and in the same time the micropatterns of the antennal basipodite caudal surface was like in the Zakarpattia population, and vice versa as shown by open circles in Figure 4.

Some of the studied individuals from Orlov Pond possessed intermediate qualitative characters (for instance the P4 intercoxal sclerite had long setules like in Zakarpattia population but the setules were sparse and arranged at an angle to the sclerite like in Dniester individuals, as was observed with quantitative characters). This is also shown by open circles in Figure 4.

As for quantitative characters (indexes) in the Orlov Pond population most of them were not observed in Ukrainian populations as shown in Figure 4 (black and open figures).

Table 5. Morphometric indexes in males *Eucyclops serrulatus* (Fischer) and *Eucyclops taiwanensis* sp. n. Mean \pm error of the mean (min–max). Feature abbreviations shown in Figure 3.

	Cauda, L/W	Cauda, IN/OUTseta	Cauda, IN/L	P4 exo Lseg/Sp1
Saint Petersburg	4.0 \pm 0.2 (3.1–4.6)	1.6 \pm 0.1 (1.0–2.1)	0.8 \pm 0.1 (0.5–1.0)	1.2 \pm 0.1 (1.0–1.5)
Dniester Liman	3.8 \pm 0.1 (3.3–4.1)	1.9 \pm 0.1 (1.7–2.1)	1.1 \pm 0.1 (0.9–1.3)	1.6 \pm 0.1 (1.5–1.8)
Taiwan	3.2 \pm 0.1 (3.0–3.5)	0.7 \pm 0.0	0.7 \pm 0.1	1.2 \pm 0.0 (1.2–1.3)

As a result generally these characters did not divide the type population into two groups. Moreover the Saint Petersburg population differs reliably from the Zakarpattia population in P4 endo Inseta/Lseg index and from the Dniester population in caudal rami index INseta/OUTseta and P4 exo Lseg/Sp1 (Table 4). Males from Orlov Pond and from the Dniester Liman were differentiated reliably only by index P4 exo Lseg/Sp1 (Table 5).

In contrast, we found few individuals with qualitative characters looking like individuals from the Dniester Liman (black rhombuses in Figure 4) and a few individuals with characters looking like individuals from the Zakarpattia region (black triangles in Figure 4). The caudal rami index INseta/OUTseta divided these two groups as well as the two Ukrainian populations. At the same time these index values differed between the Ukrainian and Orlov Pond populations.

Populations from several European and Asian localities

Analysis of the populations from Belgium (Ghent), Slovenia, Central Russia (Syctivkar), South Ural, West Siberia (Tomsk) and South Siberia (Baikal region) revealed a similar picture to Orlov Pond. The set of characters of the Ukrainian populations does not work here in most cases and only two individuals from Belgium looked more or less similar to the Zakarpattia population (Table 6). All studied individuals of these populations had characteristics of both Ukrainian populations simultaneously, or possessed intermediate characters, or characters were not observed.

The population from Taiwan

The study of the population from Taiwan revealed significant qualitative and quantitative differences with all studied *Eucyclops serrulatus* populations (Tables 4, 5). Comparative analysis was performed, including females from both Ukrainian populations, the type population from Saint Petersburg, and males from the Dniester population. As a result, we consider that the populations from Taiwan to represent a new species *Eucyclops taiwanensis* sp. n. which is described below.

Table 6. Morphological characters studied in *Eucyclops serrulatus* (Fischer) specimens from Belgium, Russia and Slovenia. Dark grey cells (and number 1) = characters similar to the Zakarpattia population; light grey cells (and number 2) = characters similar to the Dniester Liman population; white cells with italic numbers = intermediate characters; empty white cells = character not studied.

Locality	P4 Coxal plate	AIL	Cauda IN/OUTseta	P4 exo Lseg/Spl
Belgium (Ghent) 1		1	1.5	0.8
Belgium (Ghent) 2	1	2	1.2	1.3
Belgium (Ghent) 3	1	1	1.4	1.0
Belgium (Ghent) 4	1	1	1.3	0.7
Belgium (Ghent) 5		2	1.2	1.1
Russia (South Ural)		1	1.6	1.3
Slovenia		2	1.4	1.3
Russia (Sycitivkar)	2			1.2
Russia (West Siberia-Tomsk 1)	2		1.3	1.5
Russia (West Siberia-Tomsk 2)	1	2	1.3	1.2
Russia (Baikal region)	2	1	1.3	1.2

Taxonomy

Order **CYCLOPIFORMES** Starobogatov
 Family **CYCLOPIDAE** Rafinesque
 Genus *Eucyclops* Claus
Eucyclops taiwanensis sp. n.
 (Figures 5–9)

Type material

Holotype: ZIN RN 55090, female dissected on 1 slide; from Abvil Lake, Taiwan (25.0333°N, 121.6333°W), collected by V. Alekseev, 4 October 2009.

Paratypes: ZIN RN 55091/3–13, 3 males dissected on 3 slides, 9 females from Abvil Lake, Taiwan (25.0333°N, 121.6333°W), dissected on 5 slides, collected by V. Alekseev, 4 October 2009.

Description of holotype

Female (Figures 5–7). Length measurements. Total body length without caudal setae 1015 µm, with caudal setae 1494 µm. Cephalothorax 295 µm, 4 free thoracic somites: 138/59/54/48 µm. Urosoma 317 µm: genital double-somite 63 µm, 3 following abdominal somites 54/50/46 µm, caudal rami 104 µm. Cephalothorax slightly longer than wide, with maximum width close to terminal part, frontal part of cephalothorax oval (Figure 5A).

Caudal rami parallel, 4.2 times as long as wide, with longitudinal row of spinules (*serrula*) along most of outer edge of each ramus (Figure 5B).

Antennules 12-segmented, reaching middle margin of second free thoracic somite, with denticulate hyaline membrane on 10th and 11th segments and smooth hyaline

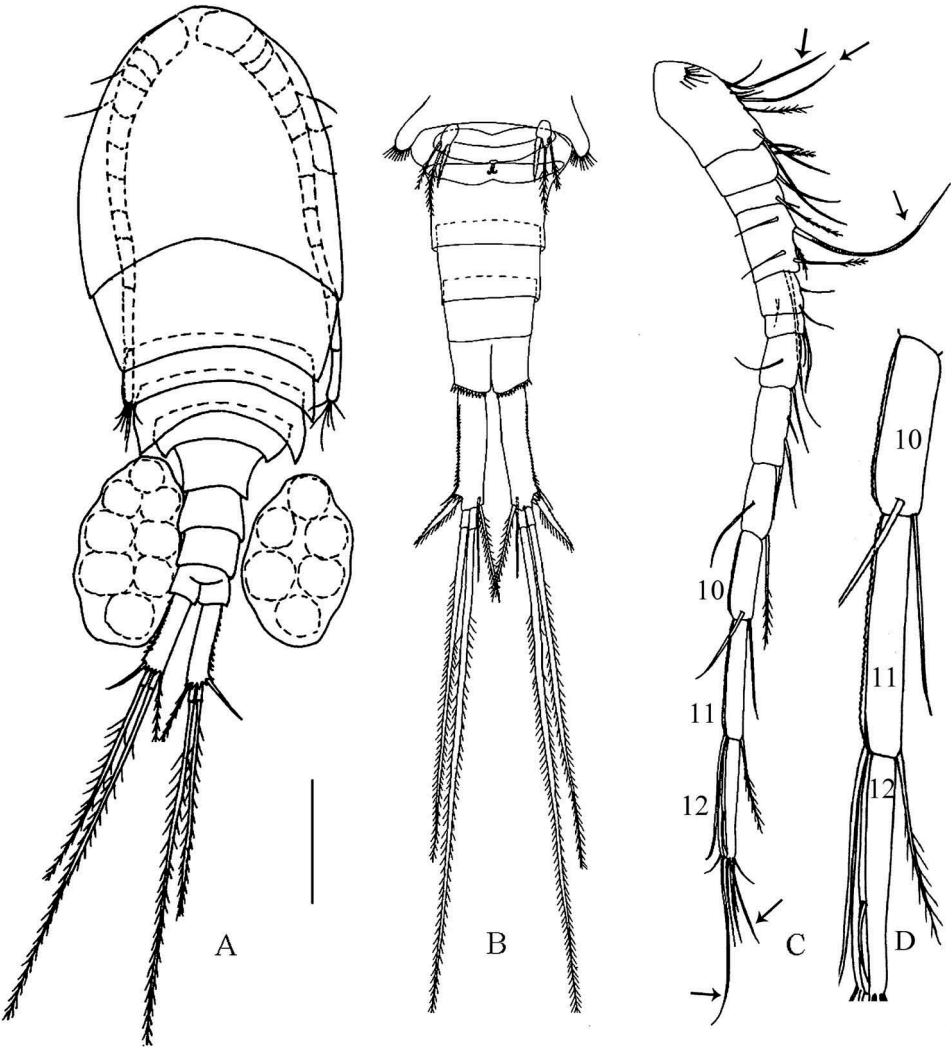


Figure 5. *Eucyclops taiwanensis* sp. n., female, ZIN RN 55090 (holotype): (A) habitus, dorsal; (B) urosome with P5, ventral side; (C) antennule with aesthetascs indicated by arrows; (D) hyaline membrane on segments 10–12 of antennule. Scale bar: A = 150 μ m; B = 100 μ m, C = 75 μ m; D = 37 μ m.

membrane on distal segment. Armament per segment as follows: 8/4/2/6/4/2/2/3/2/2/3/8. Most segments with 2 or more setae; segments 4 with 1 aesthetasc; 1 and 12 with 2 aesthetascs (Figure 5C).

Antenna (Figure 6A) with 1-segmented basipodite, 3-segmented endopodite. Exopodite represented by long, barbed seta. Antennary basipodite micro patterns (caudal face): apical group N1 consists of 3 long setules; group N2 absent, three diagonal and parallel rows of spinules (N3–5) are reduced and row N4 with gap; group of marginal spinules N17 consists of 8–10 setules.

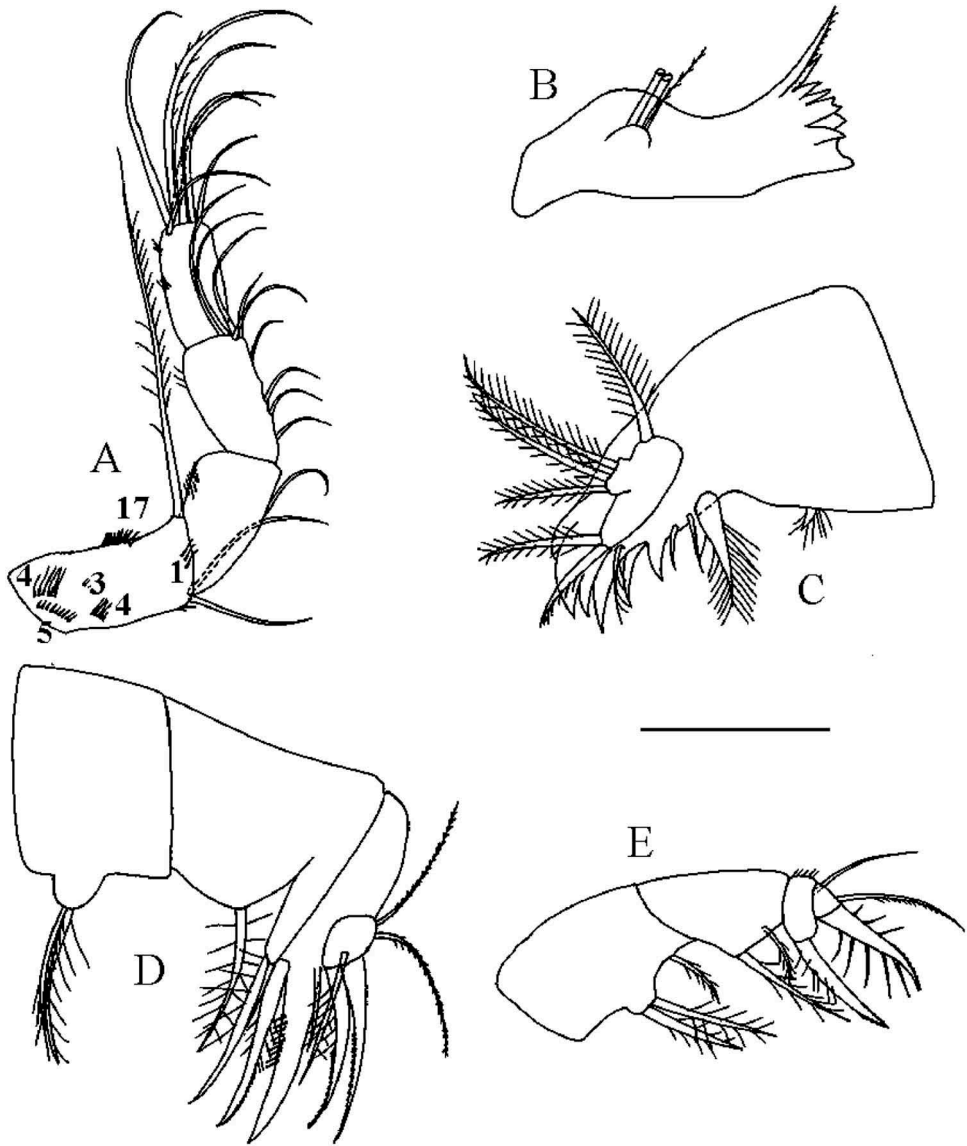


Figure 6. *Eucyclops taiwanensis* sp. n., female, ZIN RN 55090 (holotype): (A) antenna; (B) mandible; (C) maxillula; (D) maxilla; (E) maxilliped. Scale bar: A = 67 μ m, B–E = 50 μ m.

Mandible (Figure 6B) with six teeth distally, palp and rudimentary endopod with two long and one short setae.

Maxillula (Figure 6C) comprising praecoxopodite with six strong teeth and two strong setae; palp with 6 long hair-like setae of different length.

Maxilla (Figure 6D) uniramous 4-segmented: praecoxopodite with two strong median setae; syncoxopodite with two endites; proximal endite with one, distal endite with two setae; next segment with two very strong spines and small seta near to fusion of rudimentary endopodite. Latter with 2 strong spines and 3 slender setae.



Figure 7. *Eucyclops taiwanensis* sp. n., female, ZIN RN 55090 (holotype): (A) P1; (B) P2; (C) P3; (D) P4; caudal side; Scale bar: 100 μ m.

Maxilliped (**Figure 6E**) uniramous of four segments: syncoxopodite with two strong setae in middle part and 1 seta in distal part; basipodite with two setae of different length; first segment of endopodite with strong spine and hairless seta, rudiments of other endopodite segments with strong spine and 1 seta.

Swimming legs P1–4 (Figure 7) consisting of coxopodite and basipodite, bearing 3-segmented exopod and endopod. Swimming legs P1–4 spine (Arabic numerals) and seta (Roman numerals) formulae as in Table 7.

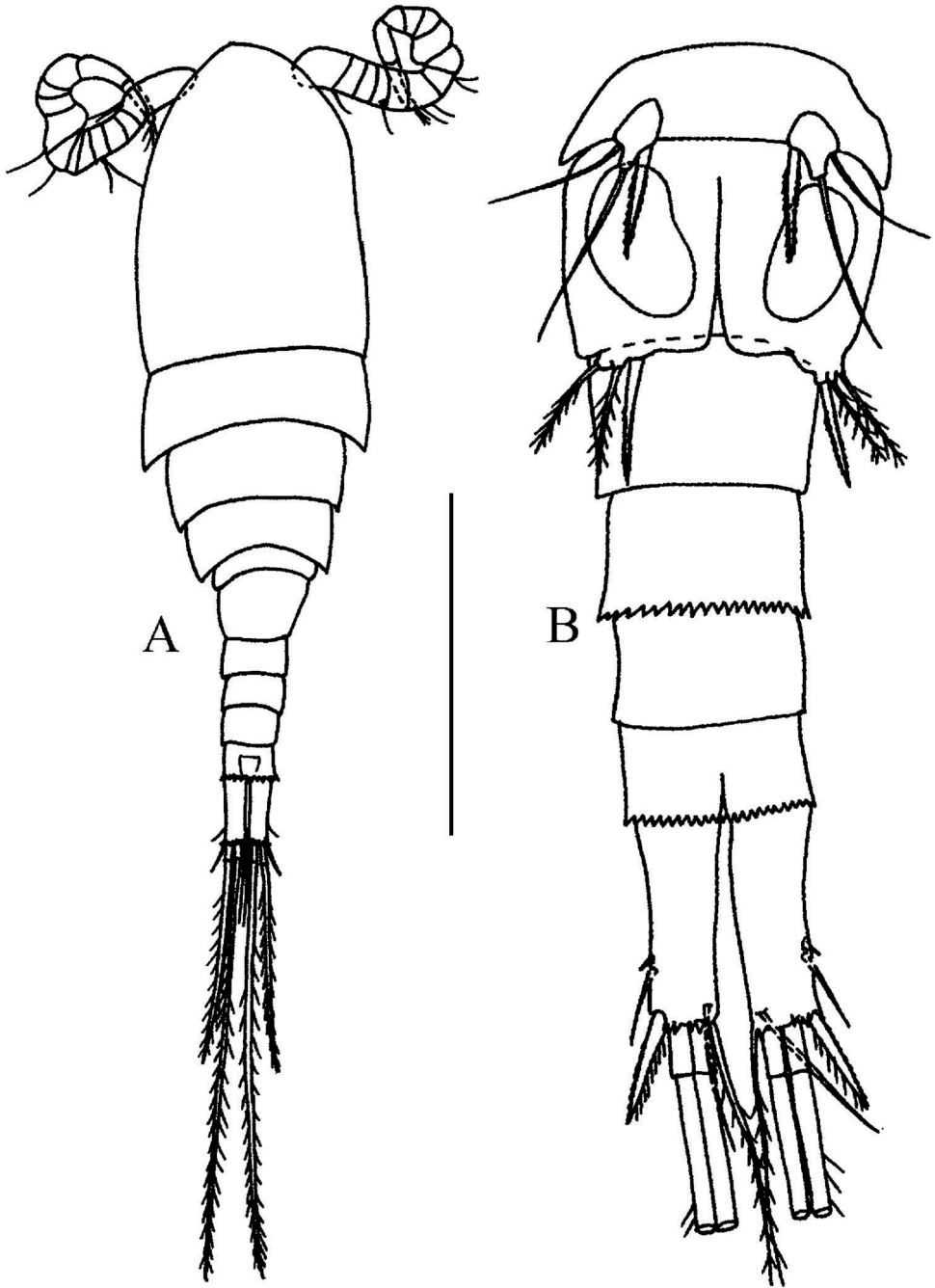


Figure 8. *Eucyclops taiwanensis* sp. n., male, ZIN RN 55091 (paratype): (A) habitus, dorsal; (B) urosome with P5 and P6, ventral side; Scale bar: A = 67 μ m, B = 100 μ m.

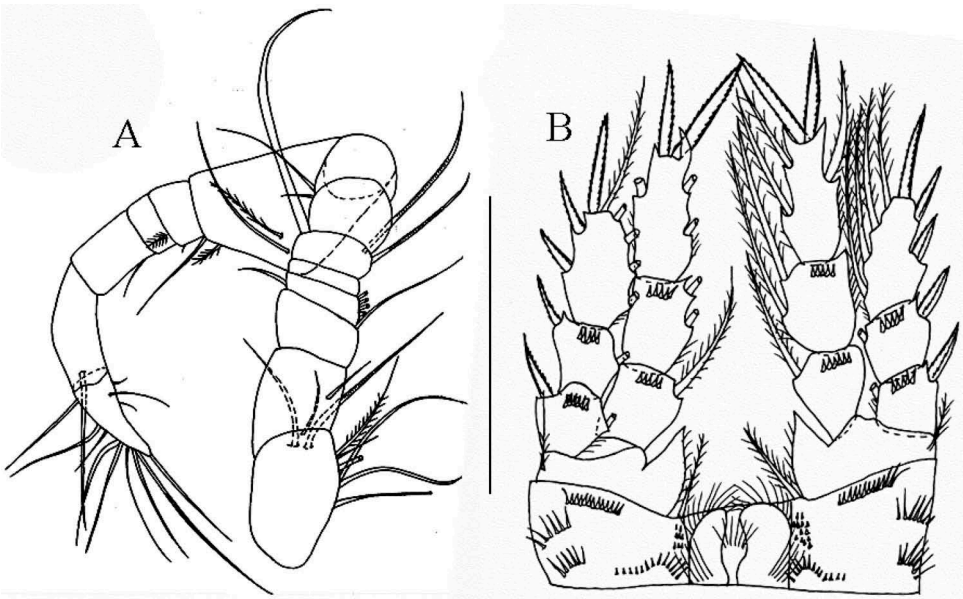


Figure 9. *Eucyclops taiwanensis* sp. n., male, ZIN RN 55091 (paratype): (A) antenna; (B) P4, caudal side. Scale bar: 100 μ m.

Table 7. Formulae of spines (Arabic numerals) and setae (Roman numerals) in swimming legs P1–4.

	Coxopodite	Basipodite	Exopodite	Endopodite
Leg 1	0–1	1–I	I–1; I–1; III–5	0–1; 0–2; 1,I,4
Leg 2	0–I	1–0	I–1; I–1; IV–5	0–1; 0–2; I,4
Leg 3	0–I	1–0	I–1; I–1; IV–5	0–1; 0–2; 1,I,4
Leg 4	0–I	1–0	I–1; I–1; III–5	0–1; 0–2; 1,II,2

Both basipodite P1 to P4 edges with long hair-like setae. Basipodite P1 with inner spine reaching middle of third segment of End (Figure 7A).

Coxopodite of P4 (Figure 7D) with strong inner spine like other legs, dense hair-like setae on inner side, and without gap among short hair-like setae on inner side. Posterior side of coxopodite with tiny spinules on inner side, not organized in groups, and groups of spinules and setules, giving formula A – I – B – E – C + D – H.

Coxopodite connected with coxal membrane. Intercoxal plate of P1–4 as in (Figure 8) with wide protuberances, but not protruding beyond free edge of plate. Coxopodites and intercoxal sclerites P1–P3 as in Figure 8.

Intercoxal plate of P4 with two groups of hair-like, long setules on free edge (Figure 7D, groups 1–2) and 3 groups of setules on plate (Figure 7D, groups 3–5). Distal spine Exp P4 about half of nearest seta and segment length (Figure 7D).

Leg P5 (Figure 6B) 1-segmented with strong knife-like inner spine and two setae; outer seta about 1.2 times as long as spine and about 1.7 times as long as medial.

Eggs packed into 2 rounded sacs.

Male (Figures 8, 9). Body length 723 μm without caudal setae (Figure 8A), and 1125 μm with caudal setae. Cephalosome 1.3 times as long as wide, with maximal width close to posterior end. Last segment of prosome smooth, last urosomal segment with row of denticles on caudal side.

Caudal rami (Figure 8B) 2.9 times longer than wide, without lateral spinules. Slender innermost caudal seta about 2.3 length of spine-like outermost caudal seta.

Antennule 14-segmented with 9 setae on first segment (Figure 9A).

Antennary basipodite basically as in female. Morphology of mouthparts and P1–3 basically as in female.

Inner edge of basipodite P4 (Figure 9B) with short setules, coxopodite of P4 with strong spine, bearing strong hair-like setae without gaps. Coxopodite of P4 with several groups of spinules A + I – B – G – C + D – H, as in female, but fewer spinules in each group. Intercoxal plate of P4 with small protuberances, strong hair-like setae on free edge, and three groups of setules on both sides. Distal segment of P4 endopodite 1.2 times longer than outer spine and 3 times longer than terminal inner seta.

P5 (Figure 8B) with inner spine 1.2 times shorter than in female, outer seta 1.2 times longer than inner spine, medial seta 1.7 times as long as spine.

P6 (Figure 8B) with inner spine and two setae with length proportions, beginning from outer seta, 1/0.85/1.

Etymology

The name of the species originates from the name of the type locality: Taiwan.

Remarks

As for intraspecific variability within the type population, we did not observe significant differences among the 13 analysed individuals. This low variability is reflected in the small standard errors of the mean in Tables 4 and 5. In general, the variation among individuals is rather small, especially for females – mean values of 0.9–2.7%. Relative standard errors of the mean for males are greater than for females, with values from 2.1 to 3.7%. The relatively high standard errors of the mean for males are most likely a result of the small sample (3 specimens).

Differential diagnosis

The Taiwanese females differed from the Zakarpattia and Dniester females simultaneously in the following indexes: caudal rami length/width (L/W); innermost caudal seta/dorsal setae (IN/DORseta); P4 distal exopodite segment length/apical spine length (P4 exo Lseg/Sp1) (Table 4).

Additionally the Taiwanese females differed from the Dniester females in: innermost caudal setae/outermost caudal setae (IN/OUTseta); P4 length of inner setae of distal endopodite segment/segment length (P4 endo Inseta/Lseg); and from the Zakarpattia females in P4 apical spine of distal endopodite segment/segment length (P4 endo Sp1/Lseg) (Table 4).

Reliable differences in caudal indexes IN/OUTseta and IN/L are shown in Table 5 and were obtained for the males from Taiwan and Dniester Liman. The Taiwanese males differed from the type population males in caudal ratio L/W (Table 5).

In general we did not observe differences in the indexes used among Taiwanese and the type populations, but the males and females of *Eucyclops taiwanensis* sp. n. differed from all studied *Eucyclops serrulatus* populations and from *E. agiloides* (G. O. Sars) in antennular hyaline membrane structure and in the coxal seta of P4 (Figure 10A–D). *Eucyclops taiwanensis* sp. n. as *Eucyclops macruroides* presents wide serrated hyaline membrane (but not so strongly serrated as in *E. denticulatus* (Graeter)) on the last

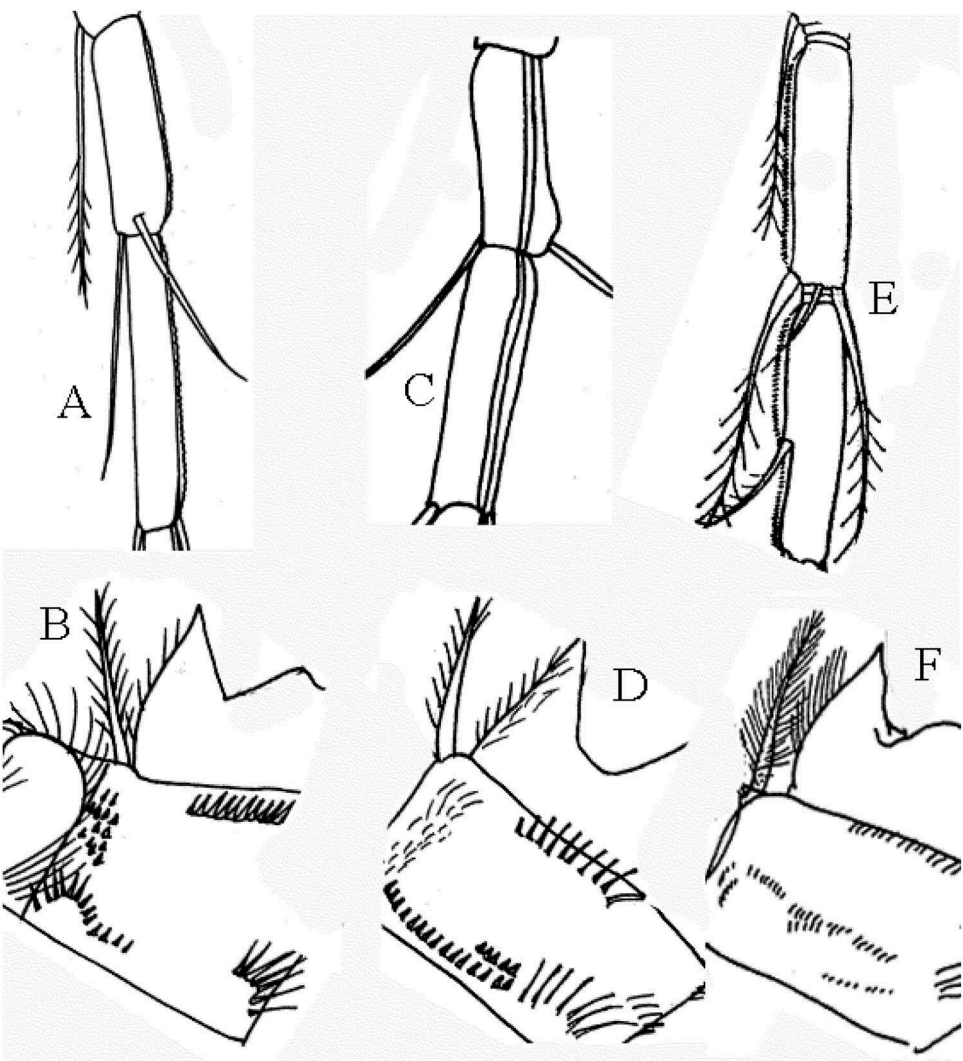


Figure 10. Hyaline membrane of antennule; (A, C) 10th–11th segments, (E) 11th–12th segments and (B, D, F) coxopodite with coxal spine P4 of (A, B) *Eucyclops taiwanensis* sp. n.; (C, D) *Eucyclops serrulatus* (Fischer); and (E, F) *Eucyclops macruroides* (Lilljeborg) according to Monchenko (1974).

antennular segment and a P4 coxal seta without a gap in setules (Figure 10A, B, E, F). The micropatterns of *Eucyclops taiwanensis* sp. n. antennary basipodite also differ from those of *Eucyclops serrulatus* as it has reduced rows of spinules (Figures 3C, D, 6A). At the same time *Eucyclops taiwanensis* sp. n. is clearly different from *E. macruioides* by a combination of characters: only antennular segments 10 and 11 have serrated hyaline membrane (not 10–12) (Figure 10A, E), and the caudal rami are significantly shorter – L/W index not more than 4.5 (not 7–9 for females and 5–6.5 for males of *E. macruioides* according to Monchenko 1974). *Eucyclops taiwanensis* sp. n. differs from *E. procerus* Dussart, 1981 in the caudal rami (L/W index 6–7).

The female of *Eucyclops taiwanensis* sp. n. can be separated from *E. macrurus* (Sars), *E. speratus* (Lilljeborg), *E. glaber* Kiefer, *E. borealis* Ishida, and *E. arcanus* Alekseev by *serrula* along most of outer edge of each caudal ramus. *Eucyclops taiwanensis* sp. n. can be separated from *E. miracle* Alekseev, *E. roseus* Ishida, *E. hadjebensis* Kiefer, *E. romaniensis* Alekseev, *E. serrulatus serrulatus* (Fischer), *E. albuferensis* Alekseev and *E. vandouwei* (Brehm) by the absence of group N2 on the posterior side of the antennal basipodite (Figure 6A).

Eucyclops taiwanensis sp. n. can also be separated from other congeneric females by the antennula with finely serrated hyaline membrane along segments 10–11, and the smooth hyaline membrane on the distal segment, in combination with other above-mentioned characters.

Discussion

The present work revealed considerable interpopulation variability in *E. serrulatus* from the studied localities.

Analysis of part of the CO1 gene revealed four clades within the studied populations. High levels of divergence among the statistically reliable clades (26.6–29.9%) could correspond to species level differences, considering previous CO1 Copepoda studies (Miracle et al. 2013; Goetze and Bradford-Grieve 2005; Sukhikh and Alekseev 2013; Sukhikh et al. 2013).

The Xucar River sequences of the clade 4 are the most distant from other sequences. This population would represent a species different of *E. serrulatus* and might be one of the recently described species of *E. serrulatus* group from the vicinity of Valencia: *E. albuferensis* Alekseev or *E. romaniensis* Alekseev. Considering the fact that the Iberian Peninsula is suggested as one of the Pleistocene refuges for freshwater organisms during glaciation periods (Miracle 1982), these sequences could also belong to one more new species, previously undescribed. Morphological analysis of this material may in future solve the question of the identity of this population.

Morphological analysis of the Taiwanese population confirmed its species status and the new species *Eucyclops taiwanensis* sp. n. is described here. The new species clearly differs from all other *E. serrulatus* populations. According to several characters, such as the caudal ramus structure and body shape, *Eucyclops taiwanensis* sp. n. seems to be part of the *serrulatus* group; however, at least two morphological characters (hyaline membrane and P4 coxal seta structure) suggest a relationship between the new species and *E. macruioides*. This relationship is confirmed by close topology of these two species sequences on the CO1 phylogenetic tree (Figure 2).

The genetic differences (27.1%) between Ukrainian populations from the Zakarpattia region and from Dniester Liman were also confirmed by morphological analysis. More surprising was that type population from Orlov Pond in Saint Petersburg is inhabited by the same two haplotypes as are present in the Ukrainian populations. Morphological heterogeneity of the type population had been observed in previous studies by Alekseev et al. (2006) who found three forms in the type population, A, B and C, differing from each other by caudal setae and swimming leg armaments. However 18S rRNA analysis did not reveal reliable differences between them (Alekseev et al. 2006). In our study, we did not find congruence between forms A, B and clades 1 and 2 using the CO1 region.

The present morphological survey of the type population using characters obtained for the Ukrainian populations also revealed some heterogeneity. Most of the studied individuals looked like a hybrid form between populations from the Dniester Liman and Zakarpattia regions, presenting characters of both populations or intermediate characters between them. Considering the distribution of haplotypes from Central Russia, Norway and France (Figure 2), we infer that this might be a possible result of colonization from different glacial refuges after the Last Glacial Maximum (LGM; 18,000 BP). Clade 1 may have been preserved in some Zakarpattia glacial refuges, as was established for some fish (Makhrov and Bolotov 2006; Costedoat and Gilles 2009), and Clade 2 may have been preserved in some glacial refuges in the Pontic region, as was observed for some fish and other organisms (Hewitt 2004; Costedoat and Gilles 2009). A potential postglacial recolonization route might have been north-eastward from these refuges, and the presence of clades 1 and 2 haplotypes together in SPb is possibly evidence of the contact zone in this region.

We found organisms looking like hybrids also in populations in Belgium, Slovenia, Central Russia, South Ural, West Siberia and Baikal region, but it is difficult to interpret the available results of genetic and morphological analyses. The addition of more material and new studies may reveal a more complicated phylogenetic structure and more clades within the studied area. The 12S rRNA and cytochrome b mitochondrial gene analysis of the populations from East European mountain lakes showed at least eight clades within *E. serrulatus* species (Hamrova et al. 2012). We suppose that new genetic forms might be present in populations from Baikal, Siberia and Slovenia. A more complete understanding will now require extended sampling and additional analysis, especially in regions of potential ancient glacial refugia.

The following conclusions can be drawn from the results in this paper:

- *Eucyclops* from Taiwan is genetically and morphologically different from European populations and is considered as a new species, *Eucyclops taiwanensis* sp. n.
- The *E. serrulatus* populations from the Dniester Liman and Zakarpattia regions represent two genetically (CO1 region) and morphologically different forms.
- The population of *terra typica* in Saint Petersburg is represented by two clades in the mitochondrial CO1 gene and the population appears morphologically to be a hybrid population.

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Disclosure statement

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